REMARKS

Reconsideration is requested.

Claims 16, 17 and 21-23 have been canceled, without prejudice.

Claims 13-15 and 18-20 are pending.

Claim 13 has been revised, without prejudice, to specify a nucleic acid comprising a sequence of SEQ ID NO: 1 or a fragment thereof containing at least 5 consecutive bases, or a nucleic acid having a complementary sequence thereto. The phrase "a functional analogue of a nucleic acid originating from another species", "a natural variant " and "a polypeptide coded by a nucleic acid" have been deleted from claim 13, without prejudice. Claim 13 further specifies that BSE is Bovine Spongiform Encephalopathy. Claim 15 has been revised to be consistent with claim 13. Claim 18 relates to a sequence of SEQ ID NO: 1.

The applicants submit that all claim amendments have been made solely in order to promote the progress of the present application and without prejudice or disclaimer. Moreover, the applicants submit that the applicants retain the right to file divisional and/or continuation applications directed to any subject matter disclosed in the present application.

The objection to the specification is traversed. The objected-to word (i.e., "encephalopathy") is not believed to be contained in the noted paragraph on page 2, lines 10-15 of the specification. Clarification is requested in the event the objection to the specification is maintained.

The objection to claims 17-19 is obviated by the above amendments. Withdrawal of the objection is requested. The claims have been revised to read on the elected subject matter.

To the extent not obviated by the above amendments, the Section 112, first paragraph "written description" , rejection of claims 13-20 is traversed. Reconsideration and withdrawal of the rejection are requested in view of the above and the following.

To the extent the rejection is based on the recitation of structural variants and analogues, functional analogues, natural variants and polypeptides (see the paragraph spanning pages 4-5 and the first two full paragraph on page 5 of the Office Action dated February 27, 2009), the above obviates the rejection. To the extent the Examiner believes that the applicants must demonstrate a "function" of SEQ ID NO:1 or "expression level" of SEQ ID NO:1 in healthy individuals, for example, to allegedly demonstrate an adequate written description (see page 4 of the Office Action dated February 27, 2009), the Examiner is urged to appreciate the description of the present specification wherein the DATAS method of marker identification is described. Specifically, DATAS identifies qualitative differences in gene expression and provides a systematic analysis of RNA splicing between two conditions : healthy/infected. DATAS leads to the identification of functionally distinct RNA variants. The DATAS method comprises three separate steps : collection of the tissue, isolation of RNA, and creation of a library containing qualitative differences and identifying novel gene fragments, which cannot be isolated by other genetic techniques.

By comparing qualitative gene expression in blood cells from healthy mammals and those infected naturally or experimentally with BSE, different signatures of genetic markers have been isolated. The naturally infected animals had terminal stage disease, whereas mammals infected by the oral route with 1 g of BSE-infected brain represent the early stage of the disease.

Implementation of the DATAS method on blood cells from cows led to the identification and isolation of several thousand genetic markers, divided into two libraries representing qualitative gene expression between healthy cows and naturally infected cows, on the one hand, and between healthy cows and experimentally infected cows, on the other hand.

The markers in these libraries were selected and validated by two approaches :

In the first approach, gene fragments common to the two DATAS libraries produced in this manner were identified. The sequences of these 11 markers are given in sequences SEQ ID NO: 16-26.

In the second approach, different clones from the two DATAS experiments were transferred to glass slides. The slides were hybridized with probes produced from biological material from naturally or experimentally infected cows and healthy cows used as controls. Through the use of two types of statistical analysis, SAM (Significance Analysis of Microarray) and PAM (Prediction Analysis of Microarray), comparing healthy versus infected animals, 15 clones were found to show a deregulation between healthy versus infected conditions. The 15 nucleic acid sequences are described in the specification as SEQ ID NO:1-15.

One of ordinary skill in the art will appreciate that the applicants were in possession of the claimed invention at the time the application was filed.

The disclosed invention provides a set of biological markers that can be used, alone or in combination(s), to detect, characterize or monitor a transmissible spongiform encephalopathy in a mammal. In particular, the disclosed invention can be used to detect the presence of prion diseases in mammalian subjects, particularly ovines, bovines and humans. The disclosed invention is particularly advantageous in that it can be carried out on live mammals, from biological fluids such as blood, plasma, platelets and the like.

Withdrawal of the Section 112, first paragraph "written description", rejection is requested.

To the extent not obviated by the above amendments, the Section 112, first paragraph "enablement" , rejection of claims 13-20 is traversed. Reconsideration and withdrawal of the rejection are requested in view of the above and the following.

The claims are submitted to be supported by an enabling disclosure. One of ordinary skill in the art will be able to make and use the claimed invention from the guidance of the specification as well as the generally advanced level of skill in the art.

The following supplementary experimental data shows by SAM and PAM analysis that SEQ ID NO:1 is specific for infected animals. Furthermore, plots of the probability density functions of the normalized log-ratios of EXB-NROA0576 (SEQ ID NO: 1) reveal a clear separation (with no overlap at all) between the expression levels of the non-infected and infected populations using this gene. Therefore, SEQ ID NO: 1

(EXB-NROA0576 gene, Homo sapiens serine/threonine kinase 24, STK24, STE20 homolog, yeast) is a marker for detecting BSE.

I. Preparation of BSE microarray slide

The aim of the microarray experiment is to identify diagnostic markers that differentiate RNA samples taken from blood of infected animals versus RNA from blood of control animals. The first step of the preparation of the microarray slide was to select which DATAS clones were going to be present on the slide. The microarray slide should contain all the clusters and singletons isolated by the DATAS experiments on naturally infected samples and on experimentally infected samples.

The clones from each cluster were chosen to cover the consensus sequences. So more than one clone could be chosen for one cluster. All the singletons were added to the list. In summary a total of 818 clones were selected in total. The 818 bacterial clones were transferred into 9 plates and grown overnight.

Mother PCRs were first carried out on the 9 glycerol plates using SP6/T7 primers The mother PCR corresponds to an amplification of bacteria from glycerol plates. The aim of the PCR is to produce material for the second PCR referred as daughter PCR. Then a daughter PCR is used to enhance specificity of the PCR product and to produce enough material for microarray dotting. The PCR products from the daughter PCR were concentrated in order to prepare material for automatic spotting.

The 818 clones selected to be spotted on a glass slide (see report August 2003) and the positive controls, microglobuline (200 ng/µl), alien 7 (Stratagene cat.No 252557) alien 10 (Stratagene cat.No 252560), alien 4 (Stratagene cat.No 252554) and alien 1

(Stratagene cat.No 252551), were spotted on glass slides. On one slide three different fields could be spotted using four different needles of the Microgrid II spotter (Biorobotics). Each field contains duplicates of each clone.

II. Preparation of hybridization probes

cRNA was produced in three steps: production of double stranded DNA followed by a clean-up phase using Phase Lock Gels (Eppendorf). Then samples were subjected to *in vitro* transcription of cRNA using a T7 promotor side (Ambion, Megascript T7 amplification kit) followed by clean-up of the cRNA produced using Rneasy mini kit (Qiagen) and subsequent quantification by Agilent electrophoresis.

All RNA were spiked with alien 1 (40 pg in the reference RNA and 10 pg in samples), alien 4 (100 pg in reference RNA and samples), alien 7 (10 pg in reference RNA and samples) and alien 10 (1 pg in reference RNA and samples). Reference RNA consists of commercial RNA of cow liver, kidney and lung in mixture of 1/3 each and for cRNA production 5 µg of each RNA sample was used as starting material.

An amino allyl labeling of cRNA with Cy3 and Cy5 (Amersham PA23001 or PA25001) and purification on Microcon Y-30 (Millipore 42410) were performed to label the probes. For this experiment, 5 µg of cRNA, labelled respectively A and B, were used as starting material. Four batches of cRNA from bovine blood RNA isolated by MagNa Pure (named M1 and M2) were included in this experiment (see report August 2003).

After labeling and purifying the cRNAs, a spectrophotometric quantification was performed to evaluate efficacy of the labeling experiment at OD 260 (measurement total

cRNA), OD 550 (measuring Cy3), OD 660 (Cy5). The frequency of incorporation of Cy3 and Cy5 was determined.

III. Hybridizations and analysis

Hybridization and statistical analyses were carried on 6 naturally infected and 5 non infected samples.

Hybridizations were performed in dye swap (CY3 and CY5 labeling) with 2µg of labelled cRNA

Normalization of the data was performed by Lowess (sub-grid) method. Genes, which passed the criteria below, were included in the analysis:

Mean log 2 ratio > 0.68 or < -0.68

Number of valid spots >1

Normalized SD <1

Methods used for statistical analysis were:

Parameters for filtering in Gene Traffic

At least 3 out of the 4 spots valid when considering dye swap

2 spots valid when considering CY3-only labeled samples

Normalization

Dye swap: self-normalization (of one swap by the other)

Samples labelled in CY3: normalization by standardization (subtraction of the mean and division by the standard deviation) of the log ratio of each slide

1.Statistical analysis

Two methods were used: the SAM (Significance Analysis of Microarrays) method which was used to identify differentially expressed genes and the PAM (Prediction Analysis of Microarrays) software, which was used to predict the status (infected or non-infected) of a cow based on its expression profile.

1.1.Results obtained by SAM

SAM was applied to the 818 genes. Among these 818 genes, STK24 gene (i.e., SEQ ID NO: 1) was identified as being the most upregulated gene (by two fold).

The following table shows the most upregulated gene, STK24 (SEQ ID NO: 1) with a low q-value (estimated false discovery rate).

Positive significant gene (Non infected > Naturally Infected)

Row	Gene Name	Score(d)	Fold Change	q-value (%)	Sequence definition
75	EXB-NROA0576	5,542317	1.95609	2,243	SEQ ID NO: 1 (of US n°10/578,672) Homo sapiens serine/threonine kinase 24 (STE20 homolog, yeast) (STK24), mRNA. 4/2003;

1.2.Results obtained by PAM.

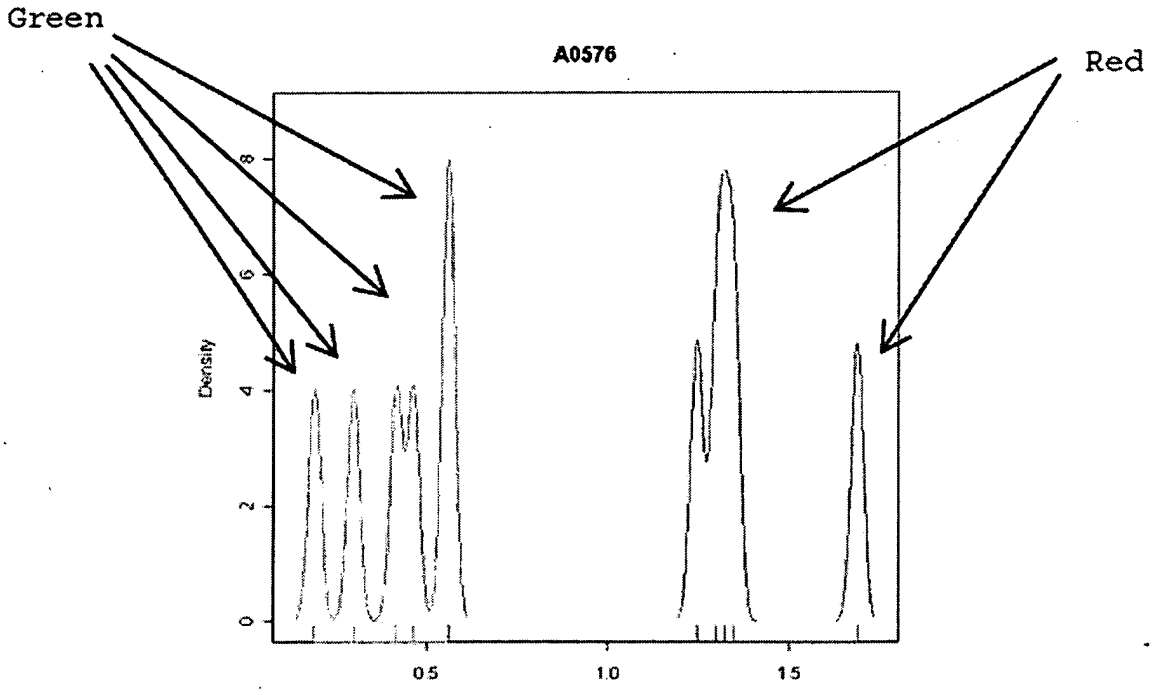
PAM was applied to the 817 genes obtained from the seventeen samples after adjustment of the data and normalization by standardization of the log-ratios of each slide. A list of the first ten genes yielding a prediction error of 1 cow over 17 (6%) was generated and the genes ranked using their absolute score. This list was crossed with the list of genes identified by SAM (section 1.1). Two were common. SEQ ID NO: 1 was the most discriminant.

The following table shows the value obtained by PAM for STK24 gene (SEQ ID NO: 1). The higher the absolute value of the scores, the more discriminant the gene is.

ID	Name	Infected score	Non infected score
SEQ ID NO: 1 (of US n°10/578,672) EXB-NROA0576	74	-0,1847	0,2078

1.3.Density probability plots

Further analysis was obtained by density probability plots. For the analysis of the naturally infected study, the plots of the probability density functions of the normalized log-ratios of EXB-NROA0576 (SEQ ID NO: 1) reveal a clear separation (with no overlap at all) between the expression levels of the non-infected and infected populations, as one can see in the figure below (where the infected population is in green and the non-infected in red) :



The applicants submit that the above further demonstrates, by SAM and PAM analysis, that SEQ ID NO:1 is specific for infected mammals. Furthermore, plots of the probability density functions of the normalized log-ratios of EXB-NROA0576 (SEQ ID NO: 1) reveal a clear separation (with no overlap at all) between the expression levels of the non-infected and infected populations using this gene. Therefore, SEQ ID NO: 1 (EXB-NROA0576 gene, Homo sapiens serine/threonine kinase 24, STK24, STE20 homolog, yeast) is a marker for detecting BSE.

Withdrawal of the Section 112, first paragraph "enablement" rejection, is requested.

The Section 112, second paragraph, rejection of claims 13 is obviated by the above amendments. Withdrawal of the rejection is requested.

The claims are submitted to be in condition for allowance and a Notice to that effect is requested. The Examiner is requested to contact the undersigned in the event anything further is required.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: /B. J. Sadoff/
B. J. Sadoff
Reg. No. 36,663

BJS:
901 North Glebe Road, 11th Floor
Arlington, VA 22203-1808
Telephone: (703) 816-4000
Facsimile: (703) 816-4100